Available online at www.sciencedirect.com
ScienceDirect
Leukemia Research 32 (2008) 1417–1423
Leukemia Research
www.elsevier.com/locate/leukres

Circumvention of glucocorticoid resistance in childhood leukemia

E.G. Haarman [a,*], G.J.L. Kaspers [a], R. Pieters [b], M.M.A. Rottier [a], A.J.P. Veerman [a,c]

[a] *Department of Pediatric Hematology/Oncology, VU University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands*
[b] *Department of Pediatric Hematology/Oncology, Erasmus University Medical Center/Sophia Children's Hospital, Dr. Molewaterplein 60, 3015 GJ Rotterdam, The Netherlands*
[c] *Dutch Childhood Oncology Group, The Hague, The Netherlands*

Received 4 November 2007; received in revised form 3 February 2008; accepted 12 February 2008
Available online 18 April 2008

Abstract

In this study, we determined if in vitro resistance to prednisolone and dexamethasone could be circumvented by cortivazol or methylprednisolone, or reversed by meta-iodobenzylguanidine in pediatric lymphoblastic and myeloid leukemia. As there were strong correlations between the LC50 values (drug concentration inducing 50% leukemic cell kill, LCK) of the different glucocorticoids and median prednisolone/methylprednisolone, prednisolone/dexamethasone and prednisolone/cortivazol LC50 ratios did not differ between the leukemia subtypes, we conclude that none of the glucocorticoids had preferential anti-leukemic activity. Meta-iodobenzylguanidine however, partially reversed glucocorticoid resistance in 19% of the lymphoblastic leukemia samples.

Keywords: Childhood leukemia; Glucocorticoid resistance; Cortivazol; Meta-iodobenzylguanidine

1. Introduction

Glucocorticoids (GCs) have been a major component of leukemia treatment protocols during the past decades [1–5]. It is of particular interest that, despite the fact that pediatric initial acute lymphoblastic leukemia (iALL) patients are treated with combination chemotherapy, both the in vivo and in vitro response to GC monotherapy are strong prognostic factors [2,6–9]. In addition, acute myeloid leukemia (AML) and relapsed ALL (rALL) patients, subgroups with a relatively poor prognosis, are highly resistant to GCs. Therefore, GCs may be important targets to improve treatment outcome. Two strategies can be used to achieve this: (1) by including more effective GCs and (2) by using compounds that enhance the cytolytic activity of GCs.

A successful example of the first strategy is the substitution of prednisone (PRD) by dexamethasone (DXM) in ALL-treatment protocols. Several studies have shown a superior relapse-free survival for DXM compared to PRD treated patients, despite the use of equivalent dosages (it was assumed that PRD and DXM have equal glucocorticoid activity if DXM is given in a 7-times lower dosage) [10–12]. These results were attributed to the more favorable pharmocokinetics of DXM: a longer duration of action and better central nervous system penetration [3,13]. In addition, DXM might be more potent than originally assumed (16× in stead of 7× more potent than PRD) [14]. This was disputed by Ito et al. who found that DXM was only 5–6× more potent than PRD in killing pediatric ALL cells in vitro [15].

Two other GCs of potential interest are methylprednisolone (mPRD) and cortivazol (CVZ). Hicsonmez demonstrated that high-dose mPRD-induced differentiation and apoptosis in pediatric AML cells [16]. They found that by using high-dose mPRD in AML-patients remission rates were improved and the duration of neutropenia was decreased. In addition, relatively high response rates (50%) were found in relapsed ALL patients (especially CNS relapse) [17]. Erduran et al. found that in children with lymphoblastic leukemia a short course of high-dose mPRD-induced apoptosis in vivo more effectively than PRD [18]. Based on their anti-inflammatory activity, mPRD is assumed to be 1.2 times as

* Corresponding author. Tel.: +31 20 4442419; fax: +31 20 4440844.
E-mail address: eg.haarman@vumc.nl (E.G. Haarman).

0145-2126/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.leukres.2008.02.009

potent as PRD. However, exact data on the relative potency of mPRD compared to PRD in pediatric leukemia samples are lacking.

CVZ is capable of inducing cell lysis in several DXM resistant cell lines including ICR27, C1, 4R4 and 3R43. This synthetic glucocorticoid is 20–50-fold more potent than DXM. In addition, CVZ is more effective than DXM in treating CNS leukemia in a SCID mouse model [19]. Recently Styczynski and co-workers demonstrated that CVZ had high anti-leukemic activity in pediatric ALL samples in vitro and might even have improved cytotoxicity in leukemia cells obtained from ALL patients with a poor in vivo prednisolone response after 7 days of prednisolone therapy [20].

A possible example of the second strategy, i.e. the addition of a compound that enhances the cytolytic activity of GCs, is meta-iodobenzylguanidine (MIBG). In a GC resistant L1210 subclone (a murine leukemia cell line) GC sensitivity was restored by incubation with MIBG. This was accompanied by an increased number of GC-binding sites and enhanced affinity of the receptor for its ligand [21]. This was thought to be the result of the inhibition of ADP-ribosylation of the glucocorticoid receptor by MIBG. So far, only one study has been published that tested the sensitising effects of MIBG on GC-induced cell kill in clinically obtained pediatric lymphoblastic leukemia samples [22].

The aims of the present study were to find out if in vitro GC resistance could be circumvented in pediatric AML and ALL cells by mPRD, CVZ, or co-incubation with MIBG.

2. Materials and methods

2.1. Leukemia samples

Leukemic samples were obtained from bone marrow or peripheral blood taken for routine diagnostic procedures with informed consent. A total of 90 iALL, 40 rALL, 32 iAML and 4 rAML samples were included in this study.

- Seventy-two iALL (common/pre-B ALL $n = 48$, T-ALL $n = 19$, pro-B ALL $n = 5$), 27 iAML, 27 rALL, 4 rAML were used to compare the in vitro cytotoxicity of PRD, DXM, mPRD and CVZ. In 31 patients (20 iALL, 7 iAML, 4 rALL) not all glucocorticoids were tested. Twenty-five patients (16 iALL, 7 iAML, 2 rALL) were only tested for PRD and CVZ. In 3 patients CVZ (1 iALL, 2 rALL), in 2 patients mPRD (2 iALL) and in 1 patient both mPRD and CVZ (1 iALL) were not evaluable due to poor duplicate experiments.
- Twelve rALL, 1 iALL and 5 iAML samples were used to test the modulating effects of MIBG on PRD resistance.
- Eighteen iALL samples and 1 rALL sample were used to determine the in vitro modulating effects of MIBG on DXM resistance.

Mononuclear samples were separated by sucrose density gradient centrifugation (Ficoll Paque, density 1.077 g/ml; Pharmacia, Sweden). The percentage of leukemic cells was determined morphologically by May-Grünwald-Giemsa (Merck, Germany) staining of cytospin preparations. When necessary, the percentage of malignant cells were enriched to >80%, using monoclonal antibodies linked to magnetic beads (Dynabeads, Norway) to eliminate contaminating cells [23].

2.2. Drugs

PRD, DXM and mPRD were obtained from the hospital pharmacy, which purchased the GCs from Hyacint (Oss, The Netherlands). Cortivazol was kindly donated by Roussel Uclaf (Paris, France). PRD and mPRD were dissolved in saline. CVZ was dissolved in ethanol. DXM phosphate was obtained as a solution ready for use. All drugs were further diluted in RPMI 1640 (Dutch modification, Gibco, Uxbridge, UK). Final drug concentrations tested are: PRD 0.016–516 μM, mPRD 0.016–534 μM, DXM 0.00047–15 μM and CVZ 0.00035–1.1 μM. Final highest ethanol concentration was 0.04%, which did not affect cell survival (data not shown).

MIBG was purchased from ICN Biopharmaceuticals Inc. (Costa Mesa, USA) and was dissolved in RPMI 1640. First, MIBG was tested as a single agent in 12 leukemia samples in six concentrations ranging from 0.038 to 1.20 μM (data not shown). MIBG concentrations inducing 10–20% LCK were selected; concentrations potent enough to have biological effects and influence cell survival, but not inducing cell kill to such an extent that modulating effects cannot be determined. For determination of the effect of MIBG on PRD cytotoxicity, 3 concentrations of PRD (10, 100 and 1000 μM) were incubated separately and in combination with 3 concentrations of MIBG (0.075, 0.15 and 0.3 μM) in triplicate. For determination of the effect on DXM cytotoxicity, 96-well microculture plates were prepared containing 20 μl aliquots of two concentrations of MIBG (0.075 and 0.15 μM) and two concentrations of DXM (0.03 and 1.9 μM) both in combination and separately in triplicate.

2.3. MTT assay

Using the MTT assay, relative leukemic cell survival (LCS) after incubation with different concentrations of a cytotoxic drug compared to the control cell survival can be calculated. Leukemic cell kill (LCK) was calculated by the equation LCK = 100% − LCS. The LC50-value, i.e. the drug concentration inducing 50% leukemic cell kill, was calculated from the dose–response curves.

The assay conditions were essentially the same as previously described [23,24]. Briefly, aliquots of 80 μl cell suspension were added to 96-well microculture plates containing 20 μl aliquots of drug solutions. Leukemic cells were incubated at 37 °C during 4 days. Eight wells with cells in medium without drugs were used to determine the control cell survival. May-Grünwald-Giemsa stained cytospins of control cells showed that all samples contained ≥70% blasts after 4 days culturing, which is required for reliable test-results [23]. Next, we added 10 μl of 5 mg/ml MTT (Sigma) to each well. The microculture plates were shaken gently for 1 min and incubated for 6 h. The yellow tetrazolium salt MTT is reduced to dark colored formazan by viable cells only. Formazan crystals were dissolved in 100 μl acidified isopropanol. The optical density (OD) was measured at 565 nm with an EL-312 microplate reader (Biotek Instruments Inc., Winooski, USA). The OD is linearly related to the number of viable cells. After correction for the optical density of the culture medium, LCS was calculated as follows: $LCS = (OD_{drug\text{-}exposed\ well})/(\text{mean } OD_{control\ wells}) \times 100\%$.